Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part A 143 (2006) 218–227
CBP
www.elsevier.com/locate/cbpa

Lysine deficiency and feed restriction independently alter cationic amino acid transporter expression in chickens (*Gallus gallus domesticus*)

Brooke D. Humphrey [a,1], Charles B. Stephensen [b], Chris C. Calvert [a], Kirk C. Klasing [a,*]

[a] *Department of Animal Science, University of California, Davis, CA 95616, USA*
[b] *U.S. Department of Agriculture Western Human Nutrition Research Center and Nutrition Department, University of California, Davis, CA 95616, USA*

Received 13 June 2005; received in revised form 22 November 2005; accepted 23 November 2005
Available online 10 January 2006

Abstract

The effect of a lysine-deficient diet on cationic amino acid transporter (CAT1-3) mRNA expression was determined in broiler chickens. Chicks consumed a lysine-adequate (LA; 1.3% lysine) or lysine-deficient (LD; 0.7% lysine) diet. Pair-fed chicks consumed the LA diet in an amount equal to that consumed by LD chicks during the previous day (PLA). CAT 1–3 mRNA expression in the liver, pectoralis and bursa of LD chicks were lower than that of LA and PLA chicks ($P<0.05$), and levels were not detectable in LD chick thymus. High affinity CAT mRNA expression in isolated bursacytes was 16-fold higher in LD chicks than that of LA chicks ($P<0.001$). Thymocyte high affinity CAT mRNA expression was 5-fold lower than that of LA chicks ($P<0.05$). The summed amount of high affinity CAT-1 and CAT-3 mRNA expression in chicks fed a lysine adequate diet was highly correlated ($r^2=0.51$; $P<0.001$) to a tissue's growth during a lysine deficiency or feed restriction. In the thymus and bursa of LD chicks, CAT mRNA levels differed between resident lymphocytes and their surrounding tissues. By expressing high affinity CAT isoforms, developing lymphocytes may have a greater ability to obtain lysine than their surrounding tissue during a lysine deficiency.

Keywords: Arginine; Lymphocyte; Lysine deficiency; System y^+

1. Introduction

Nutrient flux through the plasma membrane is permitted by nutrient transporters. These transmembrane proteins are substrate specific, kinetically defined and differentially expressed to aid in the partitioning of nutrients among tissues. Amino acids are transported across the plasma membrane by many independent systems encoded by numerous gene products, and these systems often overlap in their substrate specificities (Palacin et al., 1998). Defects in amino acid transporters can result in metabolic disorders (Wagner et al., 2001), illustrating their importance in amino acid homeostasis.

Lysine and arginine are transported by four amino acid transport systems represented by three amino acid transport families: system y^+L and $b^{0,+}$ of the glycoprotein-associated amino acid transporter (gpaAT) family, also known as the light chains of the heterodimeric amino acid transporters (HAT); system $B^{0,+}$ of the Na^+/Cl^- dependent transporter family; and the system y^+ cationic amino acid transport (CAT) family (Deves and Boyd, 1998). HATs have the greatest number and variety of amino acid transport systems identified to date in mammals and function to exchange amino acids across the plasma membrane (Chillaron et al., 2001). System y^+L and $b^{0,+}$ function in the vectorial transport of substrate in epithelial cells, and function to absorb and reabsorb cationic amino acids in the intestine and kidney, respectively (Chillaron et al., 2001). In non-epithelial cells, system y^+L can serve as an efflux pathway for cationic amino acids (Closs et al., 2004). System $B^{0,+}$ accumulates both neutral and cationic amino acids and is a specialized amino acid transporter found only in a few tissues like the lung and salivary gland (Sloan and Mager, 1999). The system y^+ CAT family is encoded by three genes (CAT1-3) producing four isoforms (CAT-1, CAT-2A, CAT-2B and CAT-3) in mammals (Deves and Boyd, 1998). A fourth gene, CAT-4,

* Corresponding author. Department of Animal Science, University of California, 1 Shields Avenue, Davis, CA 95616, USA. Tel.: +1 530 752 1901; fax: +1 530 752 0175.
E-mail address: kcklasing@ucdavis.edu (K.C. Klasing).
[1] Current address: Department of Animal and Avian Sciences, University of Maryland, 1413 Animal Science Center, College Park, MD 20742, USA.

1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2005.11.019

has been identified in humans (Sperandeo et al., 1998) but does not transport amino acids (Wolf et al., 2002). Mammalian CAT-1, CAT-2B and CAT-3 mediate high affinity transport (K_m=70–400 μM), while CAT-2A mediates low affinity transport (K_m=2–5 mM) (Closs et al., 1993a,b; Vekony et al., 2001). Unlike systems y^+L, $b^{0,+}$ and $B^{0,+}$, system y^+ is ubiquitously expressed and functions to accumulate lysine and arginine into cellular amino acid pools for use in nitrogen metabolism (Verrey et al., 2000). Therefore, the system y^+ cationic amino acid transport family is the primary amino acid transport system utilized by most tissues for lysine and arginine uptake (Broer, 2002; Closs, 2002).

Amino acid transport systems undergo adaptive regulation to changes in their substrate concentrations. The activity and expression of mammalian isoforms in vitro from system A (Gazzola et al., 2001; Ling et al., 2001), system y^+ (Aulak et al., 1999; Fernandez et al., 2001; Hyatt et al., 1997), system L (Shultz et al., 1999) and the system for excitatory amino acid transporters (EAAT) (McGivan and Nicholson, 1999) are responsive for the complete removal of individual or total amino acids. The adaptive response of amino acid transport systems in the context of a physiological setting, particularly in regards to dietary supply, is not completely understood (Broer, 2002; Brosnan, 2003; Deves and Boyd, 1998). It is known that feeding a low protein diet to pregnant rats decreases lysine and arginine uptake by placental trophoblasts and CAT-1 mRNA expression (Malandro et al., 1996), and that chick jejunal brush border membrane vesicles increase lysine transport in response to elevated dietary lysine levels (Torras-Llort et al., 1996). However, tissue specific adaptations of amino acid transporter expression to changes in dietary supply of amino acids and the consequences of these changes on tissue uptake and priorities are not known.

In a previous study, we characterized CAT isoform expression in multiple tissues of growing chicks fed a lysine-adequate diet following hatch (Humphrey et al., 2004). We determined that tissues differ in the type and amount of CAT isoform transcript that they express and infer from this that differences in CAT isoform levels may impact a tissues ability to obtain lysine and arginine should these essential amino acids become limiting (Humphrey et al., 2004). Therefore, the purpose of these experiments was to determine the effect of a dietary lysine deficiency on CAT mRNA expression in a variety of tissues and to relate these changes to tissue growth.

2. Materials and methods

2.1. Birds and management

One-day old male broiler chicks (*Gallus gallus domesticus*) (Cobb×Cobb) were raised in Petersime brooder batteries (Petersime Incubator Co., Gettysburg, OH, USA) located in an environmentally controlled room (25 °C; 18 h light:6 h darkness). At hatch, chicks were provided water and commercial chick starter for ad libitum consumption.

2.2. Diets and feeding regime

Corn–corn gluten meal–soybean meal basal diets used in all experiments are described in Table 1. Diets were formulated to meet or exceed the nutrient needs of young growing broiler chicks as suggested by NRC (1994), except for lysine. The lysine-deficient (LD) group was provided ad libitum access to a diet calculated to contain 7 g lysine-HCl/kg diet. The lysine-adequate (LA) group (controls) was provided ad libitum access to a diet containing 13 g lysine-HCl/kg diet. Lysine-HCl was supplemented to the LA diet at the expense of cellulose. Chicks fed a lysine deficient diet decrease their food intake (Webel et al., 1998), therefore a pair-fed group was provided the LA diet once a day in the amount equal to that consumed by the LD group over the previous 24 h (PLA). All experiments and procedures were approved by the UC Davis Campus Animal Care and Use Committee.

2.3. Experimental design

In all experiments, 3-day-old chicks were selected for uniform body weight from a 3-fold larger population and assigned to one of three dietary treatments. In experiment 1, each dietary treatment was fed to five replicate pens, with three chicks per pen. Tissue weight and CAT mRNA expression were determined on day 14 posthatch. In experiment 2, each dietary treatment was fed to three replicate pens, with three chicks per pen. Thymocyte and bursacyte CAT mRNA expression were determined on d 14 posthatch. In experiment 3, each dietary

Table 1
Experimental diets fed to chicks

	Lysine-adequate diet	Lysine-deficient diet
Ingredient (g/kg)		
Corn	596	596
Corn gluten meal	182	182
Soybean meal	133	133
Corn oil	38.3	38.3
Calcium phosphate	20	20
Cellulose	–	7.65
L-Lysine–HCl	7.65	–
Calcium carbonate	5.42	5.42
Sodium chloride	4.62	4.62
Arginine–HCl	3.32	3.32
DL-Methionine	1.36	1.36
L-Threonine	1.14	1.14
L-Tryptophan	0.2	0.2
Choline	0.75	0.75
Ferrous sulfate	1.01	1.01
Vitamin-mineral premix[a]	5.0	5.0
Calculated composition		
ME, mJ/kg	13.4	13.4
Crude protein, %	22.5	22.5
Crude fat, %	6.65	6.65
Available Lys, %	1.3	0.7
Available Met+Cys, %	0.99	0.99

[a] Vitamins and trace minerals were provided in the form and level described in the NRC (1994) Standard Reference Diet for Chicks.

Table 2
Primer sequences and PCR conditions for chicken CAT-1, CAT-2, CAT-3 and β-actin mRNA [a]

Gene [b]	Primer sequence	Primer mix	$MgCl_2$	PCR cycle conditions [c]	PCR product
CAT-1	5′-ATGTAGGTTGGGATGGAGCC 3′-AACGAGTAAGCCAGGAGGGT	2 μmol/L	4 mmol/L	95 °C/1 s 60 °C/4 s 72 °C/7 s 82 °C/1 s	280 bp
CAT-2	5′-GTTTCCTTCCTCATTGCTGC 3′-CCACTCCAGGCTCTTGCTAC	2 μmol/L	5 mmol/L	95 °C/1 s 62 °C/4 s 72 °C/7 s 81 °C/1 s	200 bp
CAT-3	5′-CAAGACTGGCTCTGCCTACC 3′-GGATCAACGCAAAGAAGTCC	2 μmol/L	5 mmol/L	95 °C/1 s 61 °C/3 s 72 °C/9 s 83 °C/1 s	236 bp
β-actin	5′-CTGACACCACACTTTCTACAATG 3′-GATCTTCATGAGGTAGTCCGTCAG	2 μmol/L	5 mmol/L	95 °C/1 s 63 °C/5 s 72 °C/16 s	350 bp

[a] Primer development for chicken CAT-1, CAT-2 and CAT-3 was based upon expressed sequence tags (EST) from the BBSRC Chicken EST Project with the following respective EST identification numbers: 603957065F1, 604130341F1 and 603508168F1.
[b] All PCR runs were performed with the Roche LightCycler and used an initial denaturation step at 95 °C for 120 s. PCR conditions for each primer pair consisted of 40 cycles followed by melting curve analysis by the LightCycler. The melting profile was obtained by increasing the temperature 20 °C/s from 65 °C to 95 °C while monitoring fluorescence continuously.
[c] Abbreviations: bp, base pairs; CAT, cationic amino acid transporter; s, second.

treatment was fed to four replicate pens, with three chicks per pen. Thymocyte and bursacyte CAT mRNA expression were determined on day 14 posthatch.

2.4. Tissue sampling

In experiment 1, chicks were killed by CO_2 overdose and tissues were immediately excised from each chick. The bursa (whole), liver (left lobe), thymus and pectoralis major (caudal section) from each chick were collected, blotted, weighed and immediately frozen between aluminum plates in liquid N and stored at −80 °C until further analysis.

In experiment 2, chicks were killed by CO_2 overdose on day 14 for isolation of the resident thymus and bursa cell populations. Thymus lobes from the chick's left side and the whole bursa were collected aseptically and placed into separate sterile 60 mm tissue culture plates (Corning, Corning, NY, USA) containing RPMI 1640 (Gibco Invitrogen Corporation, Carlsbad, CA, USA). Thymus and bursa tissues were minced gently with sterile forceps at ambient room temperature to release thymocyte and bursacyte populations into the media. The media from each thymocyte and bursacyte preparation was filtered through a sterile 70 μm nylon cell strainer (Falcon, Franklin Lakes, NJ, USA). Filtered thymocyte and bursacyte samples were collected in separate sterile 60 mm tissue culture plates (Corning). Minced thymus and bursa tissues were washed three times with RPMI 1640, and the media from each washing was filtered and pooled. Thymocyte and bursacyte preparations were counted and viability exceeded 95% as determined by trypan blue exclusion. Cells were centrifuged at 3000×*g* for 2 min at ambient room temperature, the supernatant was discarded, cell pellets were resuspended in RNAlater (Ambion, Austin, TX, USA) and stored at 4 °C.

2.5. RNA isolation

Total RNA was isolated using the RNAgents Total RNA Isolation System (Promega, Madison, WI, USA). Prior to RNA isolation, RNAlater was removed from thymocyte and bursacyte samples by washing in RPMI 1640 and all of the cells from the three chicks in each pen were pooled. Pooled cells were washed again and immediately processed for RNA isolation. Tissue samples from chicks of the same pen were also pooled for RNA isolation. Samples were homogenized with a Polytron grinder (Brinkman Instruments Inc., Westbury, NY, USA) and total RNA was isolated according to the manufacturer's instructions. Optical density at 260 nm was used to determine RNA concentrations.

Table 3
Growth rates and tissue masses of lysine-adequate (LA), lysine-deficient (LD) and pair-fed (PLA) chicks in experiment 1 [1,2]

	LA	LD	PLA	SEM
Body mass, g	326.93^{a}	141.47^{c}	163.80^{b}	5.63
Feed intake, g/c*d	27.47^{a}	13.27^{b}	12.27^{b}	0.55
Gain, g/c*d	23.94^{a}	7.09^{c}	9.06^{b}	0.51
Feed efficiency, g BW gain/g Feed intake	0.87^{a}	0.53^{c}	0.74^{b}	0.01
Liver mass, g	11.24^{a}	4.33^{b}	5.64^{b}	0.47
Pectoralis mass, g	13.51^{a}	3.13^{c}	5.69^{b}	0.50
Bursa mass, g	0.58^{a}	0.25^{c}	0.36^{b}	0.03
Thymus mass, g	0.56^{a}	0.20^{b}	0.26^{b}	0.04
Liver mass/BW, %	3.43^{a}	2.93^{b}	3.40^{a}	0.11
Pectoralis/BW, %	4.11^{a}	2.40^{c}	3.43^{b}	0.15
Bursa mass/BW, %	0.18^{ab}	0.17^{b}	0.22^{a}	0.01
Thymus mass/BW, %	0.20^{a}	0.14^{b}	0.15^{b}	0.01

[1] Values are means ± pooled SEM, $n=5$. Means in a row not sharing common superscripts are significantly different, $P<0.05$.
[2] BW, body mass; LA, lysine-adequate; LD, lysine-deficient; PLA, pair-fed lysine-adequate diet in an amount equal to that consumed by LD chicks over the previous 24 h.

Table 4
Relative level of CAT mRNA expression in tissues from lysine-adequate (LA), lysine-deficient (LD) and pair-fed (PLA) chicks in experiment 1 [1,2]

Gene	Treatment	Tissue			
		Liver	Pectoralis	Bursa	Thymus
CAT-1	LA	1.42[a]	1.00[a]	1.85[b]	1.12[a]
	LD	0.004[b]	0.003[b]	0.002[c]	nd
	PLA	1.47[a]	1.31[a]	5.60[a]	0.43[b]
	SEM	0.18	0.17	0.60	0.21
CAT-2	LA	0.40[b]	1.00[b]	0.001	0.18
	LD	0.001[c]	0.02[c]	nd	nd
	PLA	2.83[a]	2.52[a]	0.018	0.06
	SEM	0.41	0.13	0.007	0.05
CAT-3	LA	2.21	1.23[b]	3.65[a]	2.06
	LD	nd	0.26[c]	0.09[b]	nd
	PLA	2.72	3.39[a]	5.36[a]	2.04
	SEM	0.42	0.24	0.72	0.46

[1] Values are means±pooled SEM, $n=5$, of mRNA expression relative to pectoralis LA. Means within a column and tissue not sharing common superscripts are significantly different, $P<0.05$.
[2] CAT, cationic amino acid transporter; LA, lysine-adequate; LD, lysine-deficient; nd, not detectable; PLA, pair-fed lysine-adequate diet in an amount equal to that consumed by LD chicks over the previous 24 h.

2.6. RT-PCR

Tissue total RNA (2 μg) and bursacyte and thymocyte total RNA (1 μg) samples were reverse transcribed to cDNA and quantitative real-time PCR analysis of CAT-1, CAT-2 and CAT-3 was performed with a Roche LightCycler (Roche Diagnostics, Indianapolis, IN, USA) as previously described (Humphrey et al., 2004). Minor modification were made with the cycle conditions since primer-dimer interferes with quantification in samples containing a low amplicon concentration (Lekanne Deprez et al., 2002; Vandesompele et al., 2002). Therefore, an additional step was added to allow for measurement of fluorescence above the primer dimer melting point and below the specific product melting point (Table 2). This modification permits the fluorescence of only the desired PCR product to be quantified (Ball et al., 2003; Hein et al., 2001). Sample PCR amplification efficiencies were determined in the log-linear phase with the LinRegPCR program (Ramakers et al., 2003). Sample and reference sample C_t values were not subtracted from an endogenous control C_t value (β-actin) since β-actin expression was affected by dietary treatments in our studies ($P<0.05$). In order to determine dietary effects within a tissue, the value of the pectoralis from LA chicks served as the reference. In order to determine dietary effects within a cell population, the value of the bursacytes from LA chicks served as the reference.

2.7. Plasma corticosterone and amino acid análisis

In experiment 3, chicks ($n=4$/treatment) were bled by cardiac puncture into heparinized syringes for plasma collection. Animal handling and blood collection procedures totaled less than three minutes. Total plasma corticosterone was measured in duplicate in a single batch using a commercial ^{3}H-corticosterone radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA). Plasma samples were diluted 1:25 to ensure measurement within the linear range of the standard curve and processed according to the manufacturer's instructions.

Plasma amino acids were pre-column derivatized using the Easy-fast amino acid sample testing kit (Phenomenex, Torrance, CA, USA). Amino acid concentrations were determined by Hewlett Packard 5890 gas chromatography (Agilent, Palo Alto, CA, USA) using Chrom Perfect software (Justice Laboratory Software, Melbourne, FL, USA). Derivatization and gas chromatography were performed according to the manufacturer's instructions.

For plasma arginine determination, an equal volume of plasma was added to 15% tricarboxylic acid in ddH_2O, vortexed for 1 min and centrifuged at 14,000×g at 25 °C for 30 s. Supernatants were collected and adjusted to pH 8.5 with 6 N KOH. Supernatant arginine levels were determined by enzymatic end-point analysis according to the method of Mira de

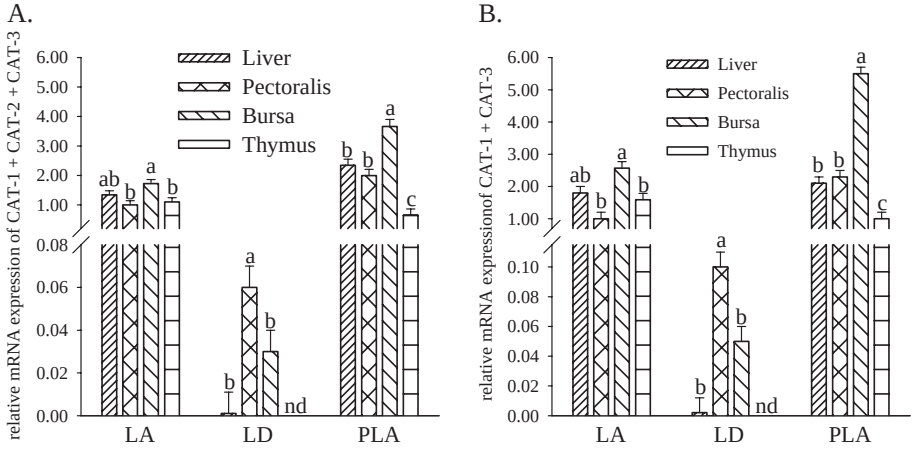

Fig. 1. Relative mRNA expression of (A) total CAT (CAT-1+CAT-2+CAT-3) and (B) high affinity CAT (CAT-1+CAT-3) in tissues from lysine-adequate (LA), lysine-deficient (LD) and pair-fed (PLA) chicks in experiment 1. Graph bars represent means±pooled SEM, $n=5$, of mRNA expression relative to pectoralis LA. Graph bars within a dietary treatment not sharing common superscripts are significantly different, $P<0.05$. Abbreviations: CAT, cationic amino acid transporter; PLA, pair-fed lysine-adequate diet in an amount equal to that consumed by LD chicks over the previous 24 h.

Orduna (2001). Absorbance was measured at optical density 350 nm and sample concentrations were determined within the linear range of an arginine standard curve.

2.8. Statistical analysis

Dependent variables were analyzed by general linear model (JMP Software, SAS Inc., Cary, NC, USA) using an analysis of variance (ANOVA). For all analysis, pen was used as the experimental unit because samples from individual birds within a pen were pooled. Data were transformed to logarithms when variances were not homogeneous (assessed with Levene's test). For experiments 1 and 3, a one-way ANOVA was used to determine the effect of treatment on each dependent variable. In experiment 2, a two-way ANOVA was used to determine the main effects of lymphocyte type, diet and their interaction for each dependent variable. When main effects or their interactions were significant ($P<0.05$), means comparisons were performed using Tukey's means comparisons. The relationship between high affinity CAT mRNA expression in LA chicks and the loss in tissue mass during a lysine deficiency was determined by linear regression using SigmaPlot (SPSS Inc, Chicago, IL, USA). Data are reported as non-transformed means and pooled standard errors.

3. Results

3.1. Growth rates and tissue weights

The effect of dietary treatments on growth rate was similar across experiments and results from experiment 1 are shown in Table 3. Body weight, feed intake, rate of gain and feed efficiency of LD chicks were lower than those of LA chicks ($P<0.05$). Body weight, rate of gain and feed efficiency were also lower for LD chicks than PLA chicks despite similar feed consumption ($P<0.05$). Liver, thymus, pectoralis and bursa weights were lower in LD chicks than in LA chicks ($P<0.05$). When organ weights were corrected for body weight (relative tissue weights), the percentage decrease in bursa, liver, thymus, and pectoralis weights due to a lysine deficiency were 5.6, 14.6, 30.0, and 41.6 compared to the chicks fed the lysine adequate diet.

3.2. Tissue CAT mRNA expression

Tissue β-actin expression was influenced by dietary treatments in tissues and cell populations in our studies ($P<0.05$). To prevent the variation of β-actin from confounding our results, tissue CAT isoform mRNA expression was compared across dietary treatments by normalizing the PCR values for each isoform in each tissue for the amount expressed in the pectoralis from LA chicks (Table 4). In order to compare functional categories of transporters, total CAT isoforms (CAT-1, CAT-2 and CAT-3) as well as high affinity CAT isoforms (CAT-1 and CAT-3) were summed and normalized for the respective amounts in pectoralis LA (Fig. 1). Total CAT and high affinity CAT mRNA expression in LA chicks was greatest in the bursa and least in the pectoralis and thymus (Fig. 1; $P<0.05$).

3.3. Lysine deficiency

LD chicks had lower CAT mRNA expression for all isoforms in all tissues compared to that of LA chicks (Table 4; $P<0.05$). The reduction in CAT mRNA expression was

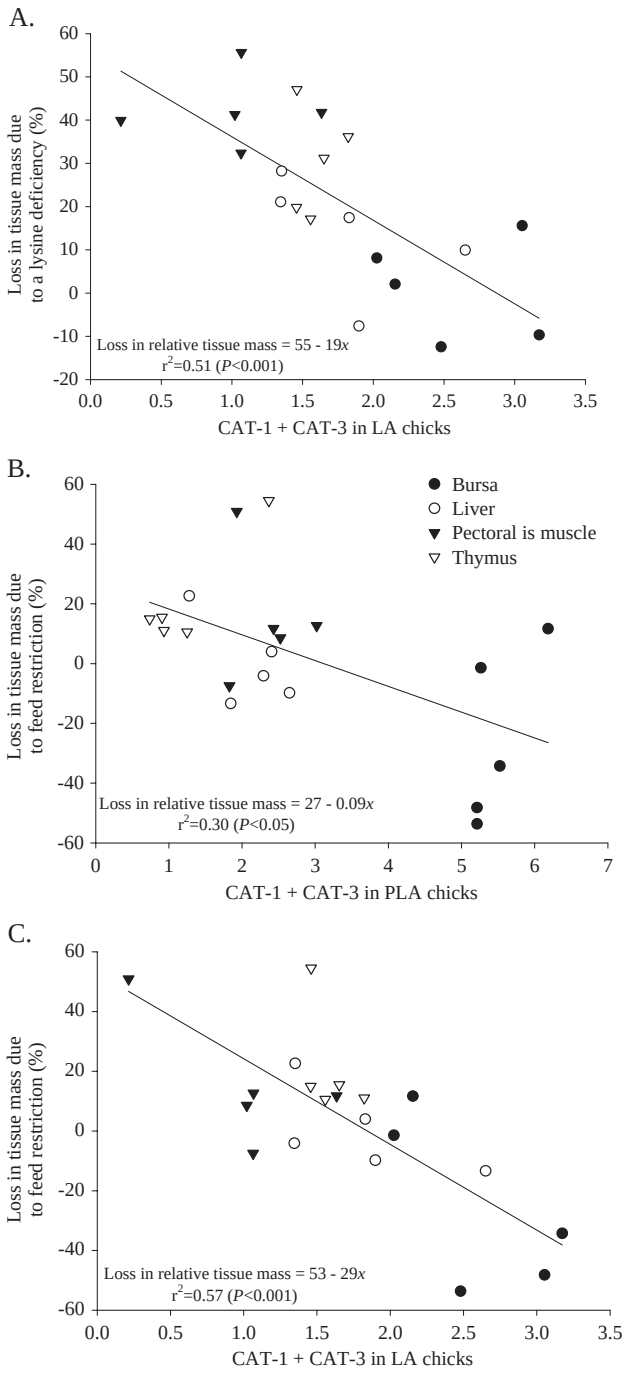

Fig. 2. The relationship between total high affinity CAT mRNA expression (CAT-1+CAT-3) and the relative loss in tissue mass due to lysine deficiency (A) or feed restriction (B and C). The loss in tissue mass due to consumption of a lysine deficient was significantly related to high affinity CAT expression in tissues of LA chicks (panel A). The loss in tissue mass due to feed restriction was significantly related to high affinity CAT expression in the tissues of those chicks that were feed restricted (PLA, panel B) and in chicks fed the lysine adequate diet (LA) ad libitum (panel C).

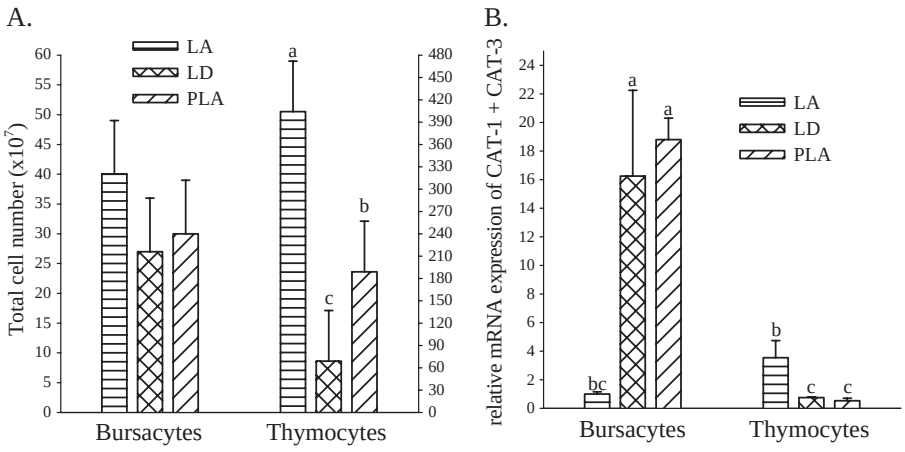

Fig. 3. (A) Numbers of bursacytes and thymocytes isolated from lysine-adequate (LA), lysine-deficient (LD) and pair-fed (PLA) chicks in experiment 2. Graph bars represent means±pooled SEM, $n=3$. Graph bars within a cell population not sharing common superscripts are significantly different, $P<0.05$. (B) Relative level of high affinity CAT mRNA expression in lymphocytes from LA, LD and PLA chicks. High affinity CAT mRNA expression is the sum of CAT-1 and CAT-3 mRNA expression within a cell population. Graph bars represent means±pooled SEM, $n=3$, of mRNA expression. Graph bars not sharing common superscripts are significantly different, $P<0.05$. Abbreviations: CAT, cationic amino acid transporter; PLA, pair-fed lysine-adequate diet in an amount equal to that consumed by LD chicks over the previous 24 h.

independent of food intake since tissue CAT isoform mRNA levels were lower in LD chicks compared to that of PLA chicks ($P<0.05$). LD chick's pectoralis CAT-1, CAT-2 and CAT-3 mRNA expression was 437-fold, 126-fold and 13-fold lower than that in PLA chicks, respectively ($P<0.05$). Thymus CAT-1, CAT-2 and CAT-3 mRNA expression was not detectable in LD chicks.

Total CAT mRNA expression in the pectoralis from LD chicks was 60-fold and 2-fold greater than that of the liver and bursa, respectively (Fig. 1A; $P<0.05$). High affinity CAT mRNA expression in the pectoralis from LD chicks was 50-fold and 2-fold greater than that of the liver and bursa, respectively (Fig. 1B; $P<0.05$). There was a highly significant negative correlation between the relative loss in tissue mass during a lysine deficiency and the level of expression of high affinity CAT in LA chicks (Fig. 2A), which can be described by the linear equation $y=0.55–0.19x$ ($r^2=0.51$; $P<0.001$).

3.4. Feed restriction

Unlike the lysine deficiency, restricting intake of a lysine adequate diet typically resulted in either no change, or greater CAT expression. The liver, pectoralis and bursa of PLA chicks all had at least one CAT isoform with greater mRNA expression than those of LA chicks. The thymus was the only tissue in PLA chicks to decrease a CAT isoform. Thymic CAT-1 mRNA expression in PLA chicks was 2.6-fold lower than in LA chicks ($P<0.05$). Total and high affinity CAT mRNA expression in PLA chicks was greatest in the bursa and least in the thymus (Fig. 1A; $P<0.05$). There was a highly significant negative correlation ($P<0.05$; $r^2=0.30$) between the loss in tissue mass due to feed restriction and the level of expression of high affinity CAT in the tissues of PLA chicks (Fig. 2B). Interestingly, there was an even stronger negative relationship ($P<0.001$; $r^2=0.57$) between tissue loss due to the feed restriction and high affinity CAT expression in LA chicks (Fig. 2C).

3.5. Bursacyte and thymocyte cell concentration

The total thymocyte numbers in LD chicks and PLA chicks were 83% and 63% lower than in LA chicks (Fig. 3A; $P<0.05$). The number of bursacytes did not change due to dietary treatments ($P>0.05$).

3.6. Lymphocyte CAT mRNA expression

In order to compare relative amounts of CAT isoform mRNA between cell populations and dietary treatments, PCR values for each isoform were normalized for the amount expressed in bursacytes from LA chicks (Table 5). CAT-1 mRNA expression did not differ between cell type ($P>0.05$)

Table 5
Relative level of CAT mRNA expression in lymphocytes from lysine-adequate (LA), lysine-deficient (LD) and pair-fed (PLA) chicks in experiment 2 [1,2]

Diet	CAT-1		CAT-3	
	Thymocytes	Bursacytes	Thymocytes	Bursacytes
LA	0.6	1.0	6.5[b]	1.0[c]
LD	0.3	0.4	1.2[c]	32[a]
PLA	0.1	0.9	1.0[c]	37[a]
SEM	0.3		5.1	
P-value: Diet	0.11		<0.01	
P-value: Cell	0.34		<0.01	
P-value: Diet×Cell	0.42		<0.01	

[1] Values are means±pooled SEM, $n=3$, of mRNA expression relative to bursacyte LA. CAT-2 mRNA expression was below the limit of detection in thymocytes and not detectable in bursacytes. Means within an isoform not sharing common superscripts are significantly different, $P<0.05$.

[2] CAT, cationic amino acid transporter; LA, lysine-adequate; LD, lysine-deficient; PLA, pair-fed lysine-adequate diet in an amount equal to that consumed by LD chicks over the previous 24 h.

or dietary treatment ($P>0.05$). CAT-2 mRNA expression was below the limit of detection in both cell types in all dietary treatments. CAT-3 mRNA was 6.5-fold higher in thymocytes than in bursacytes of LA chicks.

CAT-3 mRNA expression was very responsive to the dietary treatment ($P<0.01$). Bursacyte CAT-3 mRNA expression was 32-fold greater in LD chicks and 37-fold greater in PLA chicks compared to that in LA chicks ($P<0.05$). Thymocyte CAT-3 mRNA expression was 5.4-fold lower in LD chicks and 6.5-fold lower in PLA chicks compared to that in LA chicks ($P<0.05$).

High affinity CAT mRNA expression in bursacytes and thymocytes was dependent upon the dietary treatment (Fig. 3B; $P<0.01$). Bursacyte high affinity CAT mRNA expression was 16.2-fold greater in LD chicks and 18.8-fold greater in PLA chicks compared to that in LA chicks ($P<0.05$). Thymocyte high affinity CAT mRNA expression was 4.7-fold lower in LD chicks and 6.7-fold lower in PLA chicks compared to that in LA chicks ($P<0.05$).

3.7. Plasma amino acids and corticosterone

A lysine deficient diet resulted in decreased plasma lysine concentrations but much of this decrease was due to feed restriction (Table 6; $P<0.05$). The plasma arginine concentration was similar among dietary treatments ($P>0.05$). The total amino acid concentration and total essential amino acid concentration was greater in LD chicks than in either LA or PLA chicks ($P<0.05$). LD but not PLA chicks had higher plasma corticosterone levels than LA chicks (Table 6, $P<0.05$).

Table 6
Plasma amino acid and corticosterone concentrations in lysine-adequate (LA), lysine-deficient (LD) and pair-fed (PLA) chicks in experiment 3 [1,2]

	LA	LD	PLA	SEM
Amino Acid (μmol/L)				
Alanine	708[a]	790[a]	609[b]	21.3
Glycine	402[c]	863[a]	593[b]	26.6
Valine	236[b]	305[a]	186[c]	12.1
Leucine	454[a]	455[a]	255[b]	25.9
Threonine	740[c]	2748[a]	1966[b]	200
Serine	1142[b]	1935[a]	575[b]	186
Proline	632[ab]	860[a]	410[b]	63.8
Asparagine	21.5[b]	213[a]	11.9[b]	10.3
Aspartate	22.0	17.0	16.0	2.7
Methionine	104[a]	115[a]	47.0[b]	6.8
Isoleucine	133[a]	154[a]	95.0[b]	6.0
Glutamate	85.0	97.0	80.0	10.5
Phenylalanine	217[ab]	271[a]	128[b]	23.3
Glutamine	534	596	324	69.3
Lysine	443[a]	62.0[b]	68.0[b]	28.6
Arginine	135	168	128	26.0
Ornithine	38.0[b]	92.0[a]	9.3[c]	7.0
Histidine	50.0[ab]	19.2[b]	83.0[a]	29.8
Tryptophan	63.0[b]	76.0[a]	58.0[b]	1.6
Tyrosine	319	329	311	76.2
Total amino acids	6951[b]	10,338[a]	5911[b]	500
Total essential amino acids	3304[b]	5738[a]	3915[b]	237
Corticosterone (ng/mL)	2.9[b]	8.0[a]	3.5[b]	0.8

[1] Values are means±pooled SEM, $n=4$. Means in a row not sharing common superscripts are significantly different, $P<0.05$.

[2] LA, lysine-adequate; LD, lysine-deficient; PLA, pair-fed lysine-adequate diet in an amount equal to that consumed by LD chicks over the previous 24 h.

4. Discussion

A lysine deficiency was of interest for two primary reasons. First, this essential amino acid is often the first or second limiting amino acid in diets based on grain. Secondly, a lysine deficiency creates a nutritional environment where tissues must compete for the limited supply of this essential amino acid. The majority of lysine and arginine uptake by peripheral tissues is mediated by cationic amino acid transporters (Deves and Boyd, 1998), and quantifying their expression in tissues during a lysine deficiency could aid in determining each tissue's capacity to obtain this limiting nutrient. CAT-1 and CAT-3 mediate high affinity transport and we are assuming that CAT-2, as measured in this study, mediates low affinity, high V_{max} transport. In mammals, the CAT-2 primary transcript is alternatively spliced to produce two isoforms, CAT-2A and CAT-2B, which mediate low and high affinity transport, respectively (Closs et al., 1993a, b; Finley et al., 1995). CAT-2A is expressed in tissues requiring high rates of substrate flux, such as the liver and skeletal muscle (Closs et al., 1997, 1993b). CAT-2B is an inducible isoform whose expression is required for sustained nitric oxide production in macrophages (Nicholson et al., 2001). In our studies, total CAT-2 expression was measured since splice variants have not been identified in the chicken. Given the high expression levels of CAT-2 by skeletal muscle and liver in the present and previous studies (Humphrey et al., 2004), we were most likely measuring the equivalent of mammalian CAT-2A. CAT-1 and CAT-3 mRNA were expressed in all tissues, but CAT-2 mRNA was expressed only in the pectoralis and liver at significant levels.

4.1. Effect of diet on CAT expression

We anticipated that changes in tissue CAT expression during a lysine deficiency would be tissue dependent, with some tissues increasing their expression and others decreasing expression. However, this did not occur in our studies and CAT expression decreased precipitously in all tissues during a lysine deficiency. Tissue-wide changes in CAT expression were also observed with feed restriction, but in this case the levels for most CAT isoforms increased in many tissues.

Several factors associated with either a lysine deficiency or feed restriction may have been responsible for mediating these changes in tissue CAT mRNA expression. The lysine deficiency increased total plasma amino acid concentrations and general amino acid supply is known to regulate amino acid transport (Guidotti et al., 1978), translation initiation (Kimball, 2002), protein synthesis (Kimball and Jefferson, 2002), and expression of urea cycle (Morris, 2002) and system L mRNA (Shultz et al., 1999). Feed restriction (PLA group) did not cause a change in total plasma amino acid levels compared to ad libitum fed controls, yet their CAT expression increased in many tissues. Thus, it is unlikely that total amino acid concentrations are the dominant regulator of CAT expression. A lysine deficient diet

also caused a precipitous drop in plasma lysine concentrations and the depletion of this single amino acid may have signaled a decrease in CAT expression. In cultured cells, CAT-1 mRNA stability and translation are increased by the removal of individual amino acids (Fernandez et al., 2002; Hyatt et al., 1997). In our studies, the plasma lysine concentrations in lysine deficient and feed-restricted chicks were similar yet CAT mRNA levels often changed in opposite directions, suggesting that the low plasma lysine concentrations may not be a primary regulator of CAT expression.

A lysine-deficient diet increased plasma corticosterone concentrations independent of impaired food intake. The synthetic glucocorticoid dexamethasone decreases CAT mRNA expression in vitro (Simmons et al., 1996) and suggests that elevated corticosterone may have been an important factor responsible for the decline in CAT mRNA expression during a lysine deficiency. Altered amino acid levels and increased corticosterone may have synergistic effects on CAT mRNA expression since both of these factors regulate CAT mRNA levels (Hyatt et al., 1997; Liu and Hatzoglou, 1998). The thymus is highly sensitive to corticosterone (DePasquale-Jardieu and Fraker, 1980; Garvy et al., 1993; Laakko and Fraker, 2002), and in our study CAT mRNA levels were not detectable in this tissue when corticosterone was high. In a zinc deficiency, it is the increase in corticosterone and not the decrease in zinc supply that mediates thymic atrophy and decline in cellularity (DePasquale-Jardieu and Fraker, 1980). Taken together, changes in CAT mRNA expression during a lysine deficiency may be related to the interactive effects of decreased lysine availability, increased total amino acid availability and/or increased plasma corticosterone.

The response of all three CAT isoforms to lysine deficiency was similar in all tissues, but their response to feed restriction (PLA) was tissue and isoform dependent. For example, feed restriction decreased CAT-1 expression in the thymus, increased expression in the bursa, and did not change expression in the liver and pectoralis. Tissue specific factors involved in CAT-1 mRNA stability have been reported (Aulak et al., 1996; Yaman et al., 2002).

4.2. Plasma lysine levels and transport kinetics

Assuming that chicken CATs have transport kinetics similar to those of mammals, the plasma concentration of lysine in chicks fed a lysine adequate diet (443 μM) was above the K_m of both high affinity CAT isoforms: 100–400 μM for CAT-1 and 40–165 μM for CAT-3 (Closs et al., 1993a,b; Vekony et al., 2001). However, plasma concentrations were well bellow the K_m of CAT-2 (2–5 mM). Consequently, tissues expressing high levels of CAT-2, like the pectoralis and liver, would be expected to be able to transport high quantities of cationic amino acids in chicks consuming diets adequate in lysine. A lysine deficient diet resulted in a decrease in plasma lysine concentration to 62 μM. At this concentration, CAT-2 would not be expected to contribute much to lysine uptake and CAT-1 and CAT-3 would dominate lysine transport in chicks consuming diets deficient in lysine.

Interestingly, chicks fed a restricted amount of the lysine adequate diet had very low plasma lysine (68 μM) concentrations as well as methionine and the branched chain amino acids. Like the case of a lysine deficiency, CAT-1 and CAT-3 would be expected to mediate most of the lysine transport during feed restriction. Because CAT-1 and CAT-3 expression was maintained or even increased in many tissues during feed restriction, lysine transport would be expected to be much greater in the restricted chicks relative to the lysine deficient chicks. Thus, they apparently depleted the plasma lysine pool to the same extent as chicks fed the lysine deficient diet.

4.3. Relation between CAT expression and tissue priority

By examining the amount of CAT-1 and CAT-3 expressed in chicks fed a complete diet, we previously found that the priority for cationic amino acids was bursa > liver > thymus = pectoralis (Humphrey et al., 2004). Based on the sum of mRNA levels for the two high priority transporters (CAT-1 + CAT-3) a similar priority was found in this current study for chicks fed lysine adequate diets. If we base tissue priority on the decrease in tissue mass accreted by lysine deficient chicks relative to adequate chicks (Table 3), the priority was bursa > liver > thymus > pectoralis. Thus, the tissue expression of the high priority transporters in chicks fed a lysine adequate diet is correlated ($r^2 = 0.51$) to a tissue's growth during a lysine deficiency (Fig. 2A). The loss in relative tissue mass in tissues due to feed restriction (PLA) was also highly correlated ($r^2 = 0.57$) to expression of the high priority transporters in chicks fed a lysine adequate diet (Fig. 2C). It was also correlated ($r^2 = 0.30$) to high affinity expression in the restricted chicks (Fig. 2B). Interestingly, CAT expression in chicks fed a lysine deficient diet was not correlated to tissue growth, probably because CAT levels were minimal in all tissues at the end of the experiment.

There was a strong relationship between diet-induced changes in lymphocyte numbers and changes in their high affinity CAT mRNA levels. The decline in thymocyte numbers due to either lysine deficiency or feed restriction was associated with a decline in high affinity CAT mRNA expression. In bursacytes, high affinity CAT mRNA expression was increased by either lysine deficiency or feed restriction and total bursacyte numbers did not change. This indicates that the level of high affinity CAT mRNA expression in developing lymphocytes corresponds to and might determine their cell number.

4.4. Thymic and bursal CAT expression

The thymus and thymocytes were unique in that they decreased CAT isoform mRNA expression when feed intake was limited. The corticosterone concentration was not affected by feed restriction and can not explain this observation. It may be that decreased lysine or total amino acid supply was responsible for the down regulation of CAT isoforms, though the possibility of other hormones or nutrients can not be excluded. Regardless of the mechanism, a decreased capacity to

obtain nutrients when they become limiting may in part explain the high sensitivity of this primary immune tissue and its resident lymphocyte population to nutritional status (Savino, 2002).

CAT expression in the whole bursa and thymus differed from that of their resident lymphocytes during the lysine deficiency. The whole bursa decreased CAT expression while bursacytes increased CAT expression during the lysine deficiency. The whole thymus decreased CAT expression to undetectable levels while thymocyte CAT expression was still detectable during the lysine deficiency. The various cell types within the thymus and bursa are differentially regulated by corticosterone and this may explain the differences in CAT expression between whole tissues and their resident lymphocytes. Elevations in corticosterone mediate remodeling of the thymic stroma by decreasing epithelial volume and increasing the number and variety of proteins within the extracellular matrix (Lyra et al., 1993; Mittal et al., 1988). Corticosterone selectively induces apoptosis in developing T and B-cells undergoing gene rearrangement (King et al., 2002; Kong et al., 2002; Laakko and Fraker, 2002), resulting in a greater proportion of steroid-resistant T and B-cells residing within primary immune tissues (Compton et al., 1990; King et al., 2002; Kong et al., 2002; Laakko and Fraker, 2002). In our studies, the potential corticosterone-induced changes in lymphocyte populations and the architecture of tissue stroma may account for the differences in CAT expression between lymphocytes and their surrounding tissues during a lysine deficiency.

The lack of CAT-2 in thymocytes and bursacytes indicates that these cells exclusively utilize high affinity, low capacity lysine and arginine transporters (CAT-1 and CAT-3). Assuming similar transport kinetics between chicken and mammalian CAT systems (Torras-Llort et al., 1996, 1998), the Michaelis-Menton constants (K_m) for CAT-1 and CAT-3 are below the plasma lysine and arginine concentrations observed in all treatment groups in our studies. As a result, all CAT isoforms expressed by thymocytes and bursacytes are transporting at maximum velocity (V_{max}). The amount of total high affinity CAT isoforms expressed in developing lymphocytes was not altered by suboptimal levels of lysine, but rather by the associated reductions in food intake caused by a lysine-deficient diet. This indicates that the ability of developing lymphocytes to alter their capacity to obtain lysine may be more dependent upon food intake than the dietary level of lysine.

The fact that bursacytes increase their priority in the face of a dietary restriction while thymocytes decrease their priority may be related to the differing developmental pattern of these two cell populations. The bursa begins to regress after about 4 weeks of age and its function as an organ for diversification of the B-cell repertoire ceases as it involutes. The thymus is maintained throughout adulthood and the production of naïve T lymphocytes with novel antigen receptors is ongoing throughout the bird's life. Thus, the window for bursal function is short and loss of function during this period is likely to have a permanent detrimental effect on B lymphocyte diversity. Maintaining bursal function should be a high priority, whereas thymic function might be dispensable during periods of malnutrition because this function can be resumed later. We made measurements of CAT expression in 14-day-old chicks, which is around the time of maximal bursal function and the time when maintenance of priority would be most critical for future antibody diversity.

The ability of tissues to adapt to decreased lysine availability is reflected in the amount of high affinity CAT transporters that tissue expresses. This study indicates that transporters govern an important and often overlooked step in the inter-organ metabolism of amino acids. Determining the regulatory effects of diet on amino acid transport systems in tissues and leukocytes may help us understand how amino acids are prioritized among tissues and between different cell types within each tissue.

References

Aulak, K.S., Liu, J., Wu, J., Hyatt, S.L., Puppi, M., Henning, S.J., Hatzoglou, M., 1996. Molecular sites of regulation of expression of the rat cationic amino acid transporter gene. J. Biol. Chem. 271, 29799–29806.

Aulak, K.S., Mishra, R., Zhou, L., Hyatt, S.L., de Jonge, W., Lamers, W., Snider, M., Hatzoglou, M., 1999. Post-transcriptional regulation of the arginine transporter Cat-1 by amino acid availability. J. Biol. Chem. 274, 30424–30432.

Ball, T.B., Plummer, F.A., HayGlass, K.T., 2003. Improved mRNA quantitation in LightCycler RT-PCR. Int. Arch. Allergy Immunol. 130, 82–86.

Broer, S., 2002. Adaptation of plasma membrane amino acid transport mechanisms to physiological demands. Pflügers Arch. 444, 457–466.

Brosnan, J.T., 2003. Interorgan amino acid transport and its regulation. J. Nutr. 133, 2068S–2072S.

Chillaron, J., Roca, R., Valencia, A., Zorzano, A., Palacin, M., 2001. Heteromeric amino acid transporters: biochemistry, genetics, and physiology. Am. J. Physiol. 281, F995–F1018.

Closs, E.I., 2002. Expression, regulation and function of carrier proteins for cationic amino acids. Curr. Opin. Nephnol. Hypertens. 11, 99–107.

Closs, E.I., Albritton, L.M., Kim, J.W., Cunningham, J.M., 1993a. Identification of a low affinity, high capacity transporter of cationic amino acids in mouse liver. J. Biol. Chem. 268, 7538–7544.

Closs, E.I., Lyons, C.R., Kelly, C., Cunningham, J.M., 1993b. Characterization of the third member of the MCAT family of cationic amino acid transporters. Identification of a domain that determines the transport properties of the MCAT proteins. J. Biol. Chem. 268, 20796–20800.

Closs, E.I., Gräf, P., Habermeier, A., Cunningham, J.M., Förstermann, U., 1997. Human cationic amino acid transporters hCAT-1, hCAT-2A, and hCAT-2B: three related carriers with distinct transport properties. Biochemistry 36, 6462–6468.

Closs, E.I., Simon, A., Vekony, N., Rotmann, A., 2004. Plasma membrane transporters for arginine. J. Nutr. 134, 2752S–2759S (discussion 2765S-2767S).

Compton, M.M., Gibbs, P.S., Johnson, L.R., 1990. Glucocorticoid activation of deoxyribonucleic acid degradation in bursal lymphocytes. Poult. Sci. 69, 1292–1298.

DePasquale-Jardieu, P., Fraker, P.J., 1980. Further characterization of the role of corticosterone in the loss of humoral immunity in zinc-deficient A/J mice as determined by adrenalectomy. J. Immunol. 124, 2650–2655.

Deves, R., Boyd, C.A., 1998. Transporters for cationic amino acids in animal cells: discovery, structure, and function. Physiol. Rev. 78, 487–545.

Fernandez, J., Yaman, I., Mishra, R., Merrick, W.C., Snider, M.D., Lamers, W. H., Hatzoglou, M., 2001. Internal ribosome entry site-mediated translation of a mammalian mRNA is regulated by amino acid availability. J. Biol. Chem. 276, 12285–12291.

Fernandez, J., Yaman, I., Sarnow, P., Snider, M.D., Hatzoglou, M., 2002. Regulation of internal ribosomal entry site-mediated translation by phosphorylation of the translation initiation factor eIF2alpha. J. Biol. Chem. 277, 19198–19205.

Finley, K.D., Kakuda, D.K., Barrieux, A., Kleeman, J., Huynh, P.D., MacLeod, C.L., 1995. A mammalian arginine/lysine transporter uses multiple promoters. Proc. Natl. Acad. Sci. U. S. A. 92, 9378–9382.

Garvy, B.A., King, L.E., Telford, W.G., Morford, L.A., Fraker, P.J., 1993. Chronic elevation of plasma corticosterone causes reductions in the number of cycling cells of the B lineage in murine bone marrow and induces apoptosis. Immunology 80, 587–592.

Gazzola, R.F., Sala, R., Bussolati, O., Visigalli, R., Dall'Asta, V., Ganapathy, V., Gazzola, G.C., 2001. The adaptive regulation of amino acid transport system A is associated to changes in ATA2 expression. FEBS Lett. 490, 11–14.

Guidotti, G.G., Borghetti, A.F., Gazzola, G.C., 1978. The regulation of amino acid transport in animal cells. Biochim. Biophys. Acta 515, 329–366.

Hein, J., Schellenberg, U., Bein, G., Hackstein, H., 2001. Quantification of murine IFN-gamma mRNA and protein expression: impact of real-time kinetic RT-PCR using SYBR green I dye. Scand. J. Immunol. 54, 285–291.

Humphrey, B.D., Stephensen, C.B., Calvert, C.C., Klasing, K.C., 2004. Glucose and cationic amino acid transporter expression in growing chickens (*Gallus gallus domesticus*). Comp. Biochem. Physiol. A 138, 515–525.

Hyatt, S.L., Aulak, K.S., Malandro, M., Kilberg, M.S., Hatzoglou, M., 1997. Adaptive regulation of the cationic amino acid transporter-1 (Cat-1) in Fao cells. J. Biol. Chem. 272, 19951–19957.

Kimball, S.R., 2002. Regulation of global and specific mRNA translation by amino acids. J. Nutr. 132, 883–886.

Kimball, S.R., Jefferson, L.S., 2002. Control of protein synthesis by amino acid availability. Curr. Opin. Clin. Nutr. Metab. Care 5, 63–67.

King, L.E., Osati-Ashtiani, F., Fraker, P.J., 2002. Apoptosis plays a distinct role in the loss of precursor lymphocytes during zinc deficiency in mice. J. Nutr. 132, 974–979.

Kong, F.K., Chen, C.L., Cooper, M.D., 2002. Reversible disruption of thymic function by steroid treatment. J. Immunol. 168, 6500–6505.

Laakko, T., Fraker, P., 2002. Rapid changes in the lymphopoietic and granulopoietic compartments of the marrow caused by stress levels of corticosterone. Immunology 105, 111–119.

Lekanne Deprez, R.H., Fijnvandraat, A.C., Ruijter, J.M., Moorman, A.F., 2002. Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions. Anal. Biochem. 307, 63–69.

Ling, R., Bridges, C.C., Sugawara, M., Fujita, T., Leibach, F.H., Prasad, P.D., Ganapathy, V., 2001. Involvement of transporter recruitment as well as gene expression in the substrate-induced adaptive regulation of amino acid transport system A. Biochim. Biophys. Acta 1512, 15–21.

Liu, J., Hatzoglou, M., 1998. Control of expression of the gene for the arginine transporter Cat-1 in rat liver cells by glucocorticoids and insulin. Amino Acids 15, 321–337.

Lyra, J.S., Madi, K., Maeda, C.T., Savino, W., 1993. Thymic extracellular matrix in human malnutrition. J. Pathol. 171, 231–236.

Malandro, M.S., Beveridge, M.J., Novak, D.A., Kilberg, M.S., 1996. Rat placental amino acid transport after protein-deprivation-induced intrauterine growth retardation. Biochem. Soc. Trans. 24, 839–843.

McGivan, J.D., Nicholson, B., 1999. Regulation of high-affinity glutamate transport by amino acid deprivation and hyperosmotic stress. Am. J. Physiol. 277, F498–F500.

Mira de Orduna, R., 2001. Quantitative determination of L-arginine by enzymatic end-point analysis. J. Agric. Food Chem. 49, 549–552.

Mittal, A., Woodward, B., Chandra, R.K., 1988. Involution of thymic epithelium and low serum thymulin bioactivity in weanling mice subjected to severe food intake restriction or severe protein deficiency. Exp. Mol. Pathol. 48, 226–235.

Morris Jr., S.M., 2002. Regulation of enzymes of the urea cycle and arginine metabolism. Annu. Rev. Nutr. 22, 87–105.

Nicholson, B., Manner, C.K., Kleeman, J., MacLeod, C.L., 2001. Sustained nitric oxide production in macrophages requires the arginine transporter CAT2. J. Biol. Chem. 276, 15881–15885.

NRC, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC.

Palacin, M., Estevez, R., Bertran, J., Zorzano, A., 1998. Molecular biology of mammalian plasma membrane amino acid transporters. Physiol. Rev. 78, 969–1038.

Ramakers, C., Ruijter, J.M., Deprez, R.H., Moorman, A.F., 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. 339, 62–66.

Savino, W., 2002. The thymus gland is a target in malnutrition. Eur. J. Clin. Nutr. 56 (Suppl 3), S46–S49.

Shultz, V.D., Campbell, W., Karr, S., Hixson, D.C., Thompson, N.L., 1999. TA1 oncofetal rat liver cDNA and putative amino acid permease: temporal correlation with c-myc during acute CCl4 liver injury and variation of RNA levels in response to amino acids in hepatocyte cultures. Toxicol. Appl. Pharmacol. 154, 84–96.

Simmons, W.W., Ungureanu-Longrois, D., Smith, G.K., Smith, T.W., Kelly, R. A., 1996. Glucocorticoids regulate inducible nitric oxide synthase by inhibiting tetrahydrobiopterin synthesis and L-arginine transport. J. Biol. Chem. 271, 23928–23937.

Sloan, J.L., Mager, S., 1999. Cloning and functional expression of a human Na (+) and Cl(−)-dependent neutral and cationic amino acid transporter B(0+). J. Biol. Chem. 274, 23740–23745.

Sperandeo, M.P., Borsani, G., Incerti, B., Zollo, M., Rossi, E., Zuffardi, O., Castaldo, P., Taglialatela, M., Andria, G., Sebastio, G., 1998. The gene encoding a cationic amino acid transporter (SLC7A4) maps to the region deleted in the velocardiofacial syndrome. Genomics 49, 230–236.

Torras-Llort, M., Ferrer, R., Soriano-Garcia, J.F., Moreto, M., 1996. L-lysine transport in chicken jejunal brush border membrane vesicles. J. Membr. Biol. 152, 183–193.

Torras-Llort, M., Soriano-Garcia, J.F., Ferrer, R., Moreto, M., 1998. Effect of a lysine-enriched diet on L-lysine transport by the brush-border membrane of the chicken jejunum. Am. J. Physiol. 274, R69–R75.

Vandesompele, J., De Paepe, A., Speleman, F., 2002. Elimination of primer–dimer artifacts and genomic coamplification using a two-step SYBR green I real-time RT-PCR. Anal. Biochem. 303, 95–98.

Vekony, N., Wolf, S., Boissel, J.P., Gnauert, K., Closs, E.I., 2001. Human cationic amino acid transporter hCAT-3 is preferentially expressed in peripheral tissues. Biochemistry 40, 12387–12394.

Verrey, F., Meier, C., Rossier, G., Kuhn, L.C., 2000. Glycoprotein-associated amino acid exchangers: broadening the range of transport specificity. Pflügers Arch. 440, 503–512.

Wagner, C.A., Lang, F., Broer, S., 2001. Function and structure of heterodimeric amino acid transporters. Am. J. Physiol. 281, C1077–C1093.

Webel, D.M., Johnson, R.W., Baker, D.H., 1998. Lipopolysaccharide-induced reductions in food intake do not decrease the efficiency of lysine and threonine utilization for protein accretion in chickens. J. Nutr. 128, 1760–1766.

Wolf, S., Janzen, A., Vekony, N., Martine, U., Strand, D., Closs, E.I., 2002. Expression of solute carrier 7A4 (SLC7A4) in the plasma membrane is not sufficient to mediate amino acid transport activity. Biochem. J. 364, 767–775.

Yaman, I., Fernandez, J., Sarkar, B., Schneider, R.J., Snider, M.D., Nagy, L.E., Hatzoglou, M., 2002. Nutritional control of mRNA stability is mediated by a conserved AU-rich element that binds the cytoplasmic shuttling protein HuR. J. Biol. Chem. 277, 41539–41546.